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ALLOZYME DIVERSITY IN *AMELANCHIER ARBOREA* AND *A. LAEVIS* (ROSACEAE)

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ABSTRACT. We examined allozyme variation in five populations of *Amelanchier laevis*, which is known to be a facultative apomict in Maine, and four populations of a closely allied species, *A. arborea*. While *A. laevis* had slightly less genetic variation than *A. arborea* and less than half the number of multilocus genotypes per population, ranges of these parameters overlap extensively for the two species. Therefore, overall, these two species are comparable in the amount and distribution of their allozyme variation. Both species also had less diversity than expected based on their life history traits. Little interpopulation genetic differentiation occurred in either species, perhaps due to gene flow via avian fruit dispersal. Genetic identities are quite high among populations within each species as well as between the two species. In fact, each *A. arborea* population was more similar to at least one *A. laevis* population than to other *A. arborea* populations. *Amelanchier laevis* populations were also more similar to populations of the other species except in the case of two of the Maine populations. Recent morphological diversification and/or extensive hybridization in the genus may account for these results. Studies of additional *Amelanchier* species would indicate whether or not the level and pattern of genetic variation and the high genetic similarities of these two taxa are representative of the genus.

Key Words: *Amelanchier*, apomixis, allozymes, genetic diversity, Maloideae, Rosaceae

Among asexual woody plants, studies of allozyme variation in natural populations have focused almost exclusively on vegetatively reproducing plants (e.g., Barnes 1966, 1969; Comtois et al. 1986, 1989; Hermanutz et al. 1989; Jelinski and Cheliak 1992; Sherman-Broyles et al. 1992). Species in many angiosperm families, however, produce seeds asexually by a process called apomixis or agamospermy (Asker and Jerling 1992; Richards 1986). Yet, although several widespread woody genera have predomi-

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nantly apomictic species (e.g., *Amelanchier*, *Citrus*, *Crataegus*, *Euonymus*, *Malus*, *Mangifera*, and *Sorbus*; Richards 1986), few studies of genetic diversity in such species exist.

The maloid genera of the Rosaceae are characterized by extensive hybridization, polyploidy, and apomixis (Campbell et al. 1991; Dickinson and Campbell 1991; Phipps et al. 1991). Based on traditional views apomixis should lower genetic diversity within populations (reviewed in Asker and Jerling 1992; but see Marshall and Weir 1979 and Overath and Asmussen 1998 for a contrary view), while hybridization and polyploidy are usually associated with higher levels of genetic diversity (Moody et al. 1993). In contrast, Hamrick et al. (1992) found that woody species capable of both sexual and asexual (mainly vegetative) reproduction have as much or more allozyme diversity as sexually reproducing species. Since apomicts that have been studied in detail are capable of at least some sexual reproduction (Asker and Jerling 1992), such facultative apomicts may also fit this pattern. However, the fact that apomicts can clonally disperse long distances (via seeds) while vegetative dispersal is local (via stolons, runners, etc.) complicates the picture.

At least six maloid genera contain apomictic species (Asker and Jerling 1992; Campbell et al. 1991). In studies of morphological variation in maloid species, Campbell and Dickinson (1990) found that morphological variation in *Amelanchier*, *Crataegus*, and *Sorbus* was associated with the breeding system; apomictic species had somewhat less variation than sexual species. Although this difference was significant in their first study, it was appreciably less so when more populations were included (Dickinson and Campbell 1991). A more recent study of the amounts and distribution of morphological variation in *Amelanchier* revealed significantly more variation in the sexual species but no difference in how genetic variation was apportioned among populations (Campbell et al. 1997).

Studies of allozyme diversity in maloid species have yielded variable results. Proctor et al. (1989) found dramatic differences in a study of peroxidase diversity among *Sorbus* species in England: populations of polyploid apomicts contained almost no variation, while in diploid sexual populations genetically unique individuals could be recognized. In contrast, Aas et al. (1994) found morphological and isozyme variation, as well as evidence for sexual reproduction, in German populations of *S. latifolia*, one of

the apomictic species in the English study (Proctor et al. 1989). In a study of allozyme diversity in natural populations of several North American *Malus* species, Dickson et al. (1991) found slightly less genetic diversity than the average for long-lived woody species (Hamrick and Godt 1989). One of the species studied, *M. coronaria*, is considered an apomict (Campbell et al. 1991).

Due to apomixis, polyploidy, and hybridization the woody genus *Amelanchier* Medikus is taxonomically confusing and, thus, has been the subject of much morphological study (e.g., Campbell and Dickinson 1990; Campbell et al. 1997; Cruise 1964; Dickinson and Campbell 1991; Wiegand 1912). Campbell, with various coworkers (Campbell et al. 1985, 1987; Weber and Campbell 1989), documented facultative apomixis in several taxa in this genus. We chose to study two closely allied *Amelanchier* species, which we initially presumed to differ in reproductive mode (sexuality), *A. laevis* Wieg. and *A. arborea* (Michx. f.) Fern.

Both species are shrubs or small trees and widespread in eastern North America. Their distributions are similar and largely overlapping. *Amelanchier laevis* ranges from southern Canada to northern Georgia, while *A. arborea* ranges as far south as Florida (Gleason and Cronquist 1991). These species are similar enough morphologically that some have suggested lumping them into a single species (e.g., Cruise 1964). The main morphological differences appear to be (1) young leaves are hairy on the lower surface early in the season in *A. arborea* and glabrous in *A. laevis*, (2) leaves at anthesis are less than half-grown in *A. arborea* and half-grown in *A. laevis*, and (3) fruits are red-purple and dry in *A. arborea* and dark purple and juicy in *A. laevis* (Fernald 1950; Gleason and Cronquist 1991).

Campbell et al. (1985) documented apomixis in a Maine population of *Amelanchier laevis*, while Gorchov (1988) described *A. arborea* as sexual in Michigan. However, because these species have been described as both tetraploids (Campbell et al. 1985; Cruise 1964) and diploids (Robinson and Partanen 1980) and since most polyploid Maloideae are capable of apomixis (Campbell et al. 1991), the breeding system may differ among populations of both species. Moreover, Campbell et al. (1987) suggested that ploidy level may be associated with latitude in this genus because diploid chromosome counts come from the southern part

of the northeastern United States (e.g., New Jersey). If so, reproductive mode may also vary with latitude.

Few woody apomicts and maloid species have been studied using genetic markers such as allozymes. Our study addresses this situation by describing patterns of allozyme variation within and among populations of *Amelanchier laevis* and *A. arborea*. In addition to comparing levels and patterns of genetic variation between these two species, we examined the data for geographic patterns in the levels of genetic diversity that may be indicative of differences in ploidy level or sexuality. Finally, since allozymes have proven useful in evaluating species delineations in other maloids (e.g., *Malus*; Dickson et al. 1991), our results may also provide preliminary information concerning Cruise's (1964) proposal to combine these two taxa.

MATERIALS AND METHODS

We collected leaves and twigs from five populations of *Amelanchier laevis* and four populations of *A. arborea* from Maine to Georgia (Figure 1). For each collecting site, we contacted local experts to determine which species were present and, when possible, had their assistance in collecting the appropriate species. Given the taxonomic uncertainty associated with this genus, some genetic diversity may represent undetected taxonomic diversity in our samples. In addition, cryptic hybridization has been implicated as a source of morphological variation within *A. laevis* (Campbell et al. 1997). (Unfortunately, voucher specimens were inadvertently destroyed.) Population 3L contains some *A. canadensis* individuals, which were not included in this study, and populations 6L and 7A are actually from one area (forest around Mountain Lake Biological Laboratory) that contained both species, although generally not intermixed; the other sites appeared to contain only one species. All adult individuals of the target species were sampled in each population (except those with more than 48 individuals in which case only 48 individuals were sampled). Samples were kept on ice until returned to the laboratory where they were frozen in liquid nitrogen and ground with a mortar and pestle. Proteins were extracted from leaves with a phosphate polyvinylpyrrolidone buffer (Mitton et al. 1977). The leaf extract was absorbed onto Whatman #3 chromatography paper wicks and stored at −70°C until analysis. Using horizontal

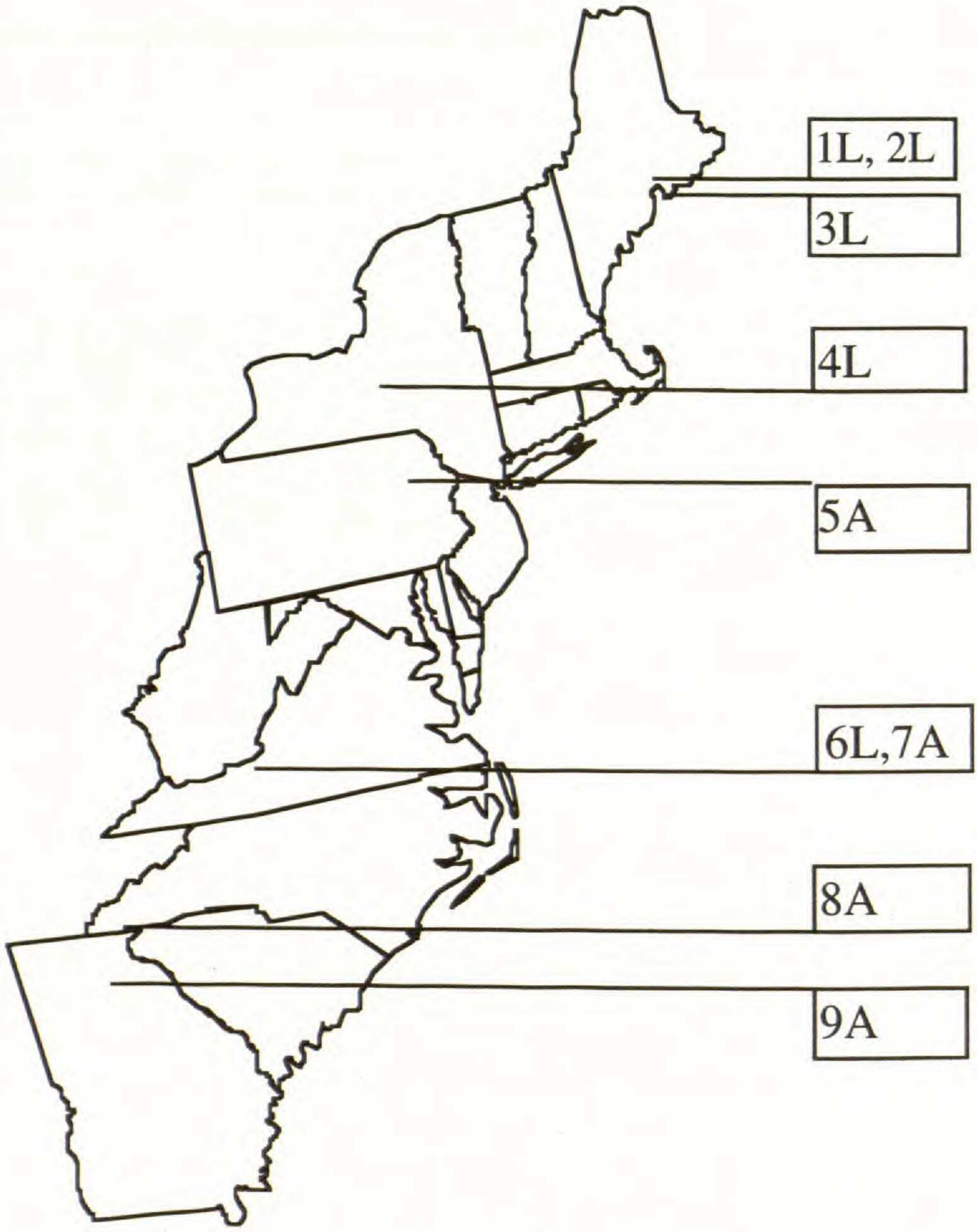

Figure 1. Locations of the nine *Amelanchier* populations sampled. Letters after the numbers refer to the species: L = *A. laevis*, A = *A. arborea*. The populations are: 1L = between two fields on Burleigh Rd., Bangor, ME; 2L = roadside on Stillwater Ave., Bangor, ME; 3L = open field on Rt. 175, near Sedgewick, ME; 4L = along trail at Hoxie Gorge, near Cortland, NY; 5A = in Schoch Heath, west of Schoch Mill Road north of Schoch Creek, in township of Penn Forest, PA; 6L and 7A = understory in forest around Mountain Lake Biological Laboratory, Mountain Lake, VA; 8A = along the Yellow Mountain Trail, Highlands Ranger District, Nantahala National Forest, near Highlands, NC; 9A = near trails in Thompson Mills Forest, Braselton, GA.

Table 1. Allozyme loci resolved for *Amelanchier laevis* and *A. arborea*. Buffer systems are from Soltis et al. (1983), 4 = Electrode buffer: 0.223 M Tris, 0.086 M citric acid, NaOH to pH 7.5; Gel buffer: 0.008 M Tris, 0.003 M citric acid, NaOH to pH 7.5; and 6 = Electrode buffer: 0.3 M boric acid, 0.1 M NaOH; Gel buffer: 0.015 M Tris, 0.003 M citric acid.

Locus	Number of Alleles		Buffer System
	A. laevis	*A. arborea*	
Aco	1	1	4
Fe-1	1	1	6
Lap	1	1	6
6-Pgd-1	2	2	4
6-Pgd-2	1	2	4
Pgi	2	3	6
Tpi-1	2	3	6
Tpi-2	1	1	6
Tpi-3	3	3	6
Tpi-4	1	4	6

starch gel electrophoresis, we resolved ten loci (Table 1) with standard recipes (Soltis et al. 1983).

We estimated the amount of genetic variation at the population and species level with the following parameters: percent polymorphic loci (P), effective number of alleles per locus (A_e), mean number of alleles per polymorphic locus (AP), and expected heterozygosity (H_e; Nei 1973). In order to estimate departure from Hardy-Weinberg expected frequencies, we also calculated observed heterozygosity (H_o) and Wright's fixation index (F) for each polymorphic locus in every population. The significance of non-zero fixation indices was tested with chi-square tests (Li and Horvitz 1953). We calculated Nei's (1973) genetic diversity statistics—total genetic diversity (H_T), mean diversity within populations (H_S), and proportion of diversity among populations (G_{ST})—over polymorphic loci to estimate variation among populations. We also estimated gene flow using the indirect methods of Wright (1951) and Slatkin (Barton and Slatkin 1986; Slatkin 1985). These statistics were calculated by a computer program (LYNSPROG) developed by M. D. Loveless and A. F. Schnabel (available from J. L. H.). We generated a UPGMA phenogram of genetic identities among all populations using NTSYS (Rohlf 1988). We also estimated multilocus genotype diversity within populations of both species by counting the number of multilocus

genotypes and computing the modified Simpson index (Pielou 1969) used by Ellstrand and Roose (1987) and more recently by Sherman-Broyles et al. (1992).

RESULTS

Although reports of tetraploids occur for both species and maloids are of polyploid origin, we saw no evidence of tetrasomic inheritance for any of the 10 loci sampled. Some enzyme systems were obviously, as expected, encoded by several loci (*Fe*, *Pgd*, and *Tpi*) and formed interlocus-heterodimers, which can be difficult to interpret. However, all loci exhibited patterns that could be interpreted with relative ease as being due to diploid inheritance. [A few loci, such as Fe-2, were not scored because they could not be read consistently. In hindsight, we might have resolved more loci by using a different extraction protocol; Overath and Kawahara (unpublished data) resolved more than 20 loci from *Amelanchier asiatica* (Sieb. & Zuc.) Endl. using a slightly different extraction buffer and grinding without liquid nitrogen.]

Genetic variation in *Amelanchier laevis* was comparable to that in *A. arborea* (Table 2). At the species level, *A. laevis* had fewer alleles per polymorphic locus (2.25 vs. 2.80 in *A. arborea*), and lower effective number of alleles per locus (1.16 vs. 1.21) and expected heterozygosity (0.090 vs. 0.116). The percent of polymorphic loci was also lower in *A. laevis* (40% vs. 60%). The same trends were seen in measures of within population variation; however, the ranges of individual population values for the two species overlap extensively. Percent polymorphic loci ranged from 20% to 30% (mean = 24%) in *A. laevis* populations and from 20% to 60% (mean = 40%) in *A. arborea* populations. The mean number of alleles per polymorphic locus ranged from 2.00 to 2.33 in *A. laevis* (mean = 2.07) and from 2.00 to 3.00 in *A. arborea* (mean = 2.35). Finally, the effective number of alleles per locus for *A. laevis* ranged from 1.07 to 1.19 (mean = 1.14) and from 1.14 to 1.22 (mean = 1.18) for *A. arborea*.

Average expected heterozygosity was also lower in populations of *Amelanchier laevis* (0.080 vs. 0.100 in *A. arborea*); however, average observed heterozygosity was slightly higher (0.071 vs. 0.066). Observed heterozygosity was lower than expected heterozygosity for both species. Consistent with the low observed het-

Table 2. Summary of allozyme diversity for ten loci within five populations of *Amelanchier laevis* and four populations of *A. arborea*. P = proportion of polymorphic loci, AP = mean number of alleles per polymorphic locus, A_e = effective number of alleles, H_o = mean observed heterozygosity, H_e = mean expected heterozygosity.

Population	Sample Size	P(%)	AP	A_e	H_o (s.d.)	H_e (s.d.)
A. laevis						
1L	30	30	2.33	1.19	0.075 (0.015)	0.101 (0.064)
2L	22	20	2.00	1.16	0.093 (0.020)	0.086 (0.060)
3L	44	20	2.00	1.17	0.086 (0.013)	0.092 (0.062)
4L	48	30	2.00	1.11	0.056 (0.011)	0.066 (0.048)
6L	17	20	2.00	1.07	0.041 (0.015)	0.054 (0.062)
Mean		24	2.07	1.14	0.071 (0.007)	0.080 (0.025)
Species level		40	2.25	1.16	—	0.090
A. arborea						
5A	48	60	2.00	1.22	0.086 (0.013)	0.118 (0.063)
7A	31	30	2.00	1.16	0.053 (0.013)	0.092 (0.057)
8A	48	50	2.40	1.20	0.094 (0.013)	0.112 (0.062)
9A	38	20	3.00	1.14	0.029 (0.009)	0.078 (0.054)
Mean		40	2.35	1.18	0.066 (0.006)	0.100 (0.030)
Species level		60	2.80	1.21	—	0.116

erozygosity, 6% of the fixation indices (F) for *A. laevis* and 17.5% for *A. arborea* were significantly greater than zero ($P \leq 0.05$).

Total genetic diversity at polymorphic loci (H_T) was higher for *Amelanchier laevis* (0.225 vs. 0.193 for *A. arborea*) indicating that allele frequencies were more skewed in *A. arborea*. Mean diversity within populations (H_S) was essentially equal for these two species (0.205 for *A. laevis* and 0.203 for *A. arborea*). Both species also had little genetic differentiation among populations (*A. laevis*, $G_{ST} = 0.054$ and *A. arborea*, $G_{ST} = 0.057$). Consequently, gene flow estimates were high for these species. Wright's estimate of gene flow was 4.41 migrants per generation for *A. laevis* and 4.17 migrants per generation for *A. arborea*. Slatkin's estimate for *A. laevis* was 12.58 migrants per generation [three private alleles, $\bar{p}(1) = 0.018$] and for *A. arborea* was 6.42 [four private alleles, $\bar{p}(1) = 0.020$].

Among *Amelanchier laevis* populations, genetic identities ranged from 0.96 to 1.00 with a mean of 0.98. Populations 3L and 2L, two of the Maine populations, were most similar and 3L

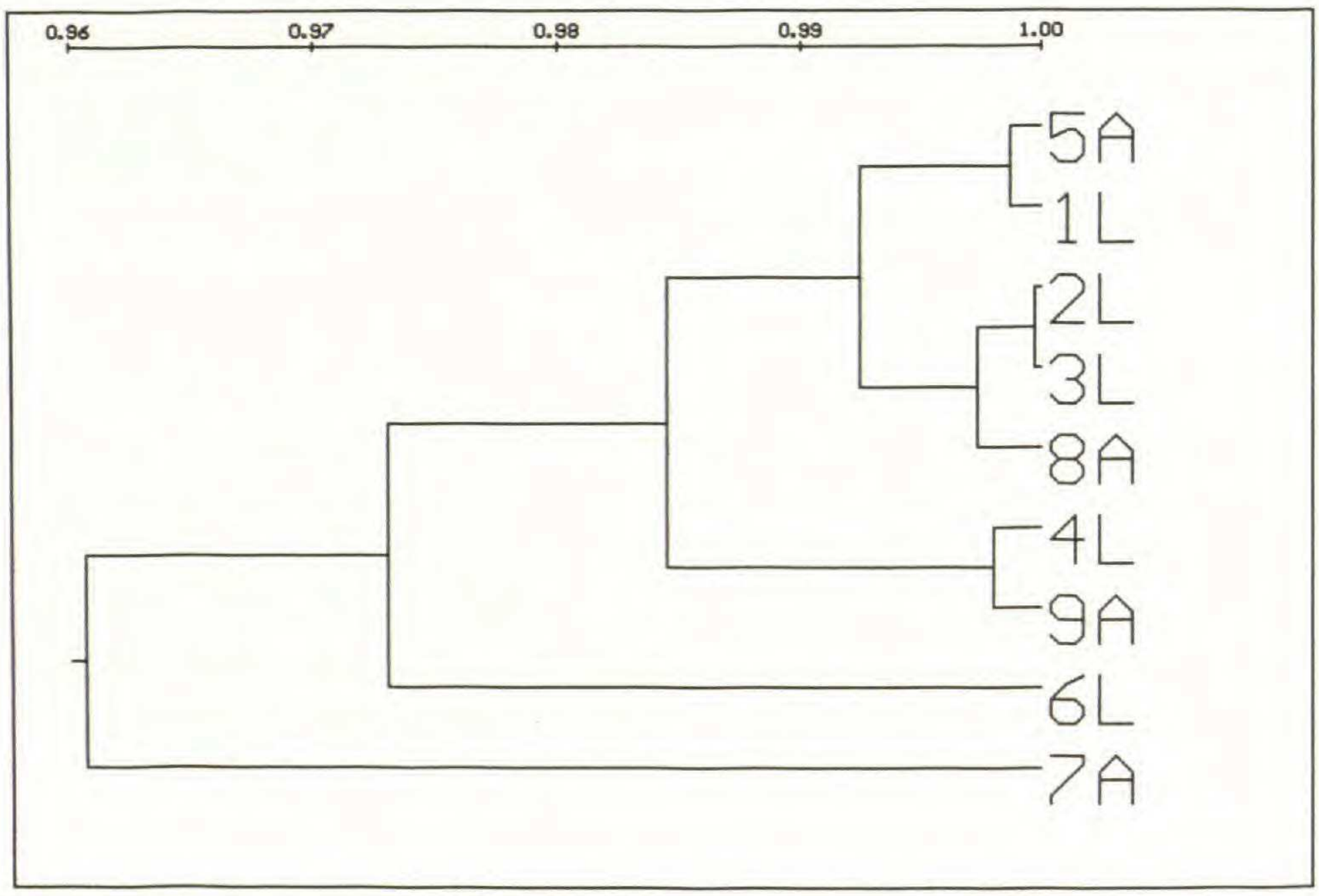

Figure 2. Phenogram of Nei's genetic identities for all nine *Amelanchier* populations. Letters after the numbers refer to the species: L = *A. laevis*, A = *A. arborea*. Locations are defined in Figure 1.

and 6L, the Virginia population, were the least similar. Genetic identities among *A. arborea* populations were similar, ranging from 0.93 to 1.00 with a mean of 0.97. The most similar *A. arborea* populations were 5A and 8A, the Pennsylvania and North Carolina populations, while the least similar were 7A and 9A, the Virginia and the Georgia populations. Genetic identity between the two species was 0.997. *Amelanchier arborea* had five alleles not found in *A. laevis* (one each at *6-Pgd-2* and *Tpi-1*, and three at *Tpi-4*). Considering all populations together (Figure 2), the two Virginia populations, 6L and 7A, were the least similar to the other populations. In all cases, *A. arborea* populations were more similar to an *A. laevis* population than to other *A. arborea* populations. *Amelanchier laevis* populations were also more similar to populations of *A. arborea* except in the case of 3L and 2L, two of the Maine populations.

The number of multilocus genotypes (MLG) per population for *Amelanchier laevis* ranged from 5 to 10 (mean = 7.4) and from 9 to 16 (mean = 12.8) for *A. arborea*. Since *A. laevis* populations tended to be smaller, differences could be due merely to sample size. When corrected for sample size (MLG/N; Table 3), *A. laevis*

Table 3. Number of multilocus genotypes (MLG), number of multilocus genotypes corrected for sample size (MLG/N), genotypic diversity indices (Dg), and probability of the most common genotype (MCG) for five populations of *Amelanchier laevis* and four populations of *A. arborea*.

Population	Sample Size	MLG	MLG/N	D_g	MCG
A. laevis					
1L	26	10	0.385	0.89	0.21
2L	19	7	0.368	0.84	0.24
3L	44	8	0.182	0.86	0.22
4L	47	7	0.149	0.77	0.35
6L	17	5	0.294	0.58	0.54
Mean (s.d.)	30.6 (14.0)	7.4 (1.81)	0.276 (0.11)	0.79 (0.12)	0.31 (0.14)
A. arborea					
5A	45	16	0.356	0.90	0.16
7A	28	9	0.321	0.87	0.29
8A	45	16	0.356	0.92	0.20
9A	38	10	0.263	0.78	0.35
Mean	39 (8.0)	12.8 (3.8)	0.324 (0.04)	0.87 (0.06)	0.25 (0.09)

still had fewer MLG's; however, the corrected values overlap more extensively than the uncorrected values. The corrected values ranged from 0.149 to 0.385 (mean = 0.276) for *A. laevis* and 0.263 to 0.356 (mean = 0.324) for *A. arborea*. Genotypic diversity indices ranged from 0.58 to 0.89 (mean = 0.79) and from 0.78 to 0.92 (mean 0.87) for *A. laevis* and *A. arborea*, respectively. The probability of the most common genotype per population for each species ranged from 0.21 to 0.54 (mean = 0.31) for *A. laevis* and from 0.16 to 0.35 (mean = 0.25) for *A. arborea*.

DISCUSSION

Overall, levels and distribution of genetic diversity in *Amelanchier laevis* and *A. arborea* are similar. Although *A. arborea* has more polymorphic loci and somewhat higher expected heterozygosity and *A. laevis* has higher mean total genetic diversity (H_T) at polymorphic loci, other measures of genetic diversity are similar. Compared to other woody species, both *Amelanchier* species have less genetic diversity (H_e; Hamrick et al. 1992).

Both *Amelanchier laevis* and *A. arborea* also have within population genetic diversity values (H_e) between the averages for

outcrossing-animal dispersed species and mixed mating-animal dispersed woody species (Hamrick et al. 1992). Similarly for both species, genetic diversity at the species level is comparable to mixed mating-animal dispersed species (Hamrick et al. 1992). Among *Amelanchier* species, sexuality is usually associated with self-incompatibility while facultative apomicts are apparently self-compatible (Campbell and Dickinson 1990; Campbell et al. 1985, 1987; Weber and Campbell 1989). Robinson (1982) tentatively concluded that *A. arborea* is self-incompatible. Campbell et al. (1985) stated that *A. laevis* is self-compatible; however, studies utilizing genetic markers are needed to determine whether any offspring are actually produced via selfing. Our data suggest that selfing may be possible in these species because some loci had a significant deficit of heterozygotes for both species (as indicated by the significantly positive fixation indices).

These two taxa have lower than average genetic diversity for woody species capable of both sexual and asexual reproduction or than is usual for sexually reproducing woody species (Hamrick et al. 1992). While it is possible that *Amelanchier* species in general have less allozyme diversity than is normal for woody species, a preliminary study of an Asian species (*A. asiatica*) over a very small geographic range in Japan found much higher levels of heterozygosity (H_e = 0.130 within populations, H_e = 0.168 at the species level; Overath and Kawahara, unpublished data). Perhaps DNA-based markers would reveal higher amounts of variation, as has been the case for *Rubus* spp. (Antonius and Nybom 1994; Nybom and Schaal 1990).

If the two American species had different ploidy levels, we might expect the polyploid to have more variation (Moody et al. 1993); however, as already mentioned, these species have both been identified as tetraploids (Campbell et al. 1985; Cruise 1964), although diploid counts have also been reported (Robinson and Partanen 1980). Since polyploidy is associated with apomixis in the Maloideae (Campbell et al. 1991), the ability to reproduce apomictically may be present and variable in both species. Maine populations of *Amelanchier laevis*, which are known to be polyploid and capable of apomixis (C. S. Campbell, pers. comm.), have slightly more genetic diversity than other *A. laevis* populations; however, no such north-south trend exists for *A. arborea*.

If ploidy and sexuality vary with latitude as Campbell et al. (1991) tentatively suggest, they have little effect on levels of allo-

zyme diversity maintained within populations of these two species. Furthermore, for the allozyme loci sampled, we found no evidence of polysomic inheritance for either species; therefore, other methods such as chromosome counts, cytodensitometry (Campbell et al. 1989), or flow cytometry (De Rocher et al. 1990) over the range of these species will be necessary to resolve this issue. Studies using genetic markers to estimate mating systems would also be helpful in determining whether variation in sexuality occurs and whether it accompanies variation in ploidy level.

G_{ST} values for both species are low, indicating little genetic differentiation among populations. If most reproduction is asexual and plants are isolated, we might expect to see higher levels of population differentiation than in purely sexual species which, presumably, would have more gene flow, at least via pollen. However, G_{ST} is marginally lower and gene flow (Nm) is slightly higher in *Amelanchier laevis*, the only one of the two species in which apomixis has been documented. Furthermore, G_{ST} for both species is similar to the mean G_{ST} for woody species capable of both sexual and asexual reproduction (G_{ST} = 0.051; Hamrick et al. 1992). Not surprisingly, mean G_{ST} for species whose seeds are ingested is also similar (0.051; Hamrick et al. 1992). Fruits of *Amelanchier* are attractive to birds (Gorchov 1988), which may contribute to gene flow and, thereby, lower population differentiation. If seed migration is extensive, we would expect that populations of a highly apomictic species, for which seeds are clones, to be more similar than those of a sexual species.

Both *Amelanchier laevis* and *A. arborea* have fewer multilocus genotypes per population than the average for clonal species (mean = 16.1; Ellstrand and Roose 1987), but higher indices of genotypic diversity (Dg = 0.62; Ellstrand and Roose 1987). Most of the species Ellstrand and Roose (1987) considered were not apomictic; however, if we consider only the apomictic species, the trends stay the same (mean MLG = 29.1 and Dg = 0.58). Ellstrand and Roose (1987) also found that sampling additional polymorphic loci increased the number of multilocus genotypes per population. Since the number of loci resolved (10) and genetic diversity at these loci are relatively low, clonal diversity may be underestimated. In any case, *A. laevis* has approximately half the number of multilocus genotypes as *A. arborea*, which is a sexual species in at least part of its range (Michigan; Gorchov 1988). However, this difference is due mainly to larger sample sizes (due

to larger population sizes) for *A. arborea* as indicated by the ratio of multilocus genotype/sample size (MLG/N; Table 3).

When comparing genetic identity values, we find that these two taxa are highly similar (I = 0.997). Populations within each species also have high genetic identities as do populations of both taxa combined. In fact, most populations of *Amelanchier laevis* are more similar to *A. arborea* populations than to other populations of *A. laevis* (Figure 2). If ploidy levels and/or sexuality vary among populations in these species, perhaps those populations that group together are of the same ploidy level. Dickson et al. (1991) explained high genetic identities among three *Malus* taxa (I = 0.983 to 0.996) as a possible example of rapid speciation because morphological divergence may have occurred faster than that of allozymes. Similar types of evidence are emerging for other groups of species in which hybridization is extensive (Hodges and Arnold 1994).

In mixed populations of these two *Amelanchier* species, Cruise (1964), using a hybrid index, found more than 25% of the individuals had intermediate morphological characters. He suggested that *A. arborea* and *A. laevis* should be combined with a third species, *A. canadensis* (L.) Medikus, in which apomixis has been documented (Campbell et al. 1987). Genetic identities between these taxa and others in the genus would be useful in gauging similarity among *Amelanchier* species in general. If genetic identities between *A. laevis* and *A. arborea* prove to be high compared to those of other congeners, Cruise's (1964) proposal to combine these two species would be warranted. If, however, *Amelanchier* species in general have high genetic identities, perhaps these species have recently undergone a rapid morphological divergence, as has been proposed for *Malus* (Dickson et al. 1991) and other plant species (Hodges and Arnold 1994). A less likely explanation, if these species are predominantly apomictic throughout their range, is that widespread hybridization may prevent species divergence.

If these two species differ in mating system, our results would support those of Dickinson and Campbell's (1991) morphological study of *Amelanchier laevis* and *A. bartramiana* (Tausch) Roemer. *Amelanchier laevis*, the facultative apomict, has slightly lower, but basically comparable, amounts of variation than that in its sexual congener. However, recently in a more extensive study, Campbell et al. (1997) found that the sexual species had more

morphological variation, although, as in our study, the distribution of variation among populations did not differ.

Because sexuality may vary within both taxa, more in-depth studies of *Amelanchier* species, including documentation of mating systems for each population, are needed. Geographic variation in sexuality would make *Amelanchier* ideal for studying the effects of facultative apomixis within a single taxon by comparing totally sexual and facultatively apomictic populations. In addition, studies of the amounts of apportionment of variation, as measured by genetic markers such as allozymes, for other *Amelanchier* taxa are needed to ascertain whether the high genetic identity between *A. laevis* and *A. arborea* is due to the fact that these two species should be merged or that little genetic differentiation among congeners is characteristic of this genus.

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LITERATURE CITED

Aas, G., J. Maier, M. Baltisberger, and S. Metzger. 1994. Morphology, isozyme variation, cytology, and reproduction of hybrids between *Sorbus aria* (L.) Crantz and *S. torminalis* (L.) Crantz. Botanica Helvetica 104: 195–214.

Antonius, K. and H. Nybom. 1994. DNA fingerprinting reveals significant genetic variation in a wild raspberry *Rubus idaeus* population. Molecular Ecology 3: 177–180.

Asker, S. E. and L. Jerling. 1992. Apomixis in Plants. CRC Press, Boca Raton, FL.

Barnes, B. V. 1966. The clonal growth habit of American aspens. Ecology 47: 439–447.

———. 1969. Natural variation and delineation of clones of *Populus tre-*

muloides and *P. grandidentata* in northern lower Michigan. Silvae Genet. 18: 130–142.

BARTON, N. H. AND M. SLATKIN. 1986. A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. Heredity 56: 409–415.

CAMPBELL, C. S. AND T. A. DICKINSON. 1990. Apomixis, patterns of morphological variation, and species concepts in subfam. Maloideae (Rosaceae). Syst. Bot. 15: 124–135.

———, C. W. GREENE, AND S. E. BERQUIST. 1987. Apomixis and sexuality in three species of *Amelanchier*, shadbush (Rosaceae, Maloideae). Amer. J. Bot. 74: 321–328.

———, ———, AND T. A. DICKINSON. 1991. Reproductive biology in subfam. Maloideae (Rosaceae). Syst. Bot. 16: 333–349.

———, ———, B. F. NEUBAUER, AND J. M. HIGGINS. 1985. Apomixis in *Amelanchier laevis*, shadbush (Rosaceae, Maloideae). Amer. J. Bot. 72: 1397–1403.

———, R. T. RIDING, AND W. A. WRIGHT. 1989. Nuclear DNA levels in megagametophytes of a sexual and an apomictic species of *Amelanchier* (Rosaceae, Maloideae) and their putative hybrid. Amer. J. Bot. 76 (6, Suppl.): 230.

———, W. A. WRIGHT, T. F. VINING, AND W. A. HALTEMAN. 1997. Morphological variation in sexual and agamospermous *Amelanchier* (Rosaceae). Canad. J. Bot. 75: 1166–1173.

COMTOIS, P., S. PAYETTE, AND J. P. SIMON. 1989. Similitude génétique et mode de dispersion: la natur des populations clonales de peupliers baumiers (*Populus balsamifera* L.) au Nouveau-Québec. Canad. J. Bot. 67: 1208–1215.

———, J. P. SIMON, AND S. PAYETTE. 1986. Clonal constitution and sex ratio in northern populations of balsam poplar, *Populus balsamifera*. Holarc. Ecol. 9: 251–260.

CRUISE, J. F. 1964. Studies of natural hybrids in *Amelanchier*. Canad. J. Bot. 42: 651–663.

DE ROCHER, E. J., K. R. HARKINS, D. W. GALBRAITH, AND H. J. BOHNERT. 1990. Developmentally regulated systematic endopolyploidy in succulents with small genomes. Science 250: 99–101.

DICKINSON, T. A. AND C. S. CAMPBELL. 1991. Population structure and reproductive ecology in the Maloideae (Rosaceae). Syst. Bot. 16: 350–362.

DICKSON, E. E., S. KRESOVICH, AND N. F. WEEDEN. 1991. Isozymes in North American *Malus* (Rosaceae): Hybridization and species differentiation. Syst. Bot. 16: 363–377.

ELLSTRAND, N. C. AND M. L. ROOSE. 1987. Patterns of genotypic diversity in clonal plants. Amer. J. Bot. 74: 123–131.

FERNALD, M. L. 1950. Gray's Manual of Botany. 8th ed. American Book Co., New York.

GLEASON, H. A. AND A. CRONQUIST. 1991. Manual of Vascular Plants of Northeastern United States and Adjacent Canada, 2nd ed. The New York Botanical Garden, Bronx, NY.

GORCHOV, D. L. 1988. Effects of pollen and resources on seed number and

other fitness components in *Amelanchier arborea* (Rosaceae: Maloideae). Amer. J. Bot. 75: 1275–1285.

HAMRICK, J. L. AND M. J. W. GODT. 1989. Allozyme diversity in plant species, pp. 43–63. *In*: A. H. D. Brown, M. T. Clegg, A. L. Kahler, and B. S. Weir, eds., Plant Population Genetics, Breeding, and Genetic Resources. Sinauer Associates, Sunderland, MA.

———, ———, AND S. L. SHERMAN-BROYLES. 1992. Factors influencing levels of genetic diversity in woody plant species. New Forests 6: 95–124.

HERMANUTZ, L. A., D. J. INNES, AND I. M. WEIS. 1989. Clonal structure of arctic dwarf birch (*Betula glandulosa*) at its northern limit. Amer. J. Bot. 76: 755–761.

HODGES, S. A. AND M. L. ARNOLD. 1994. Columbines—a geographically widespread species flock. Proc. Nat. Acad. Sci. USA 91: 5129–5232.

JELINSKI, D. E. AND W. M. CHELIAK. 1992. Genetic diversity and spatial subdivision of *Populus tremuloides* (Salicaceae) in a heterogeneous landscape. Amer. J. Bot. 79: 728–736.

LI, C. C. AND D. G. HORVITZ. 1953. Some methods of estimating the inbreeding coefficient. Amer. J. Hum. Genet. 5: 107–117.

MARSHALL, D. R. AND B. S. WEIR. 1979. Maintenance of genetic variation in apomictic plant populations. I. Single locus models. Heredity 42: 159–172.

MITTON, J. B., Y. B. LINHART, J. L. HAMRICK, AND J. S. BECKMAN. 1977. Observations on the genetic structure and mating system of ponderosa pine in the Colorado Front Range. Theor. Appl. Genet. 51: 5–13.

MOODY, M. E., L. D. MUELLER, AND D. E. SOLTIS. 1993. Genetic variation and random drift in autotetraploid populations. Genetics 134: 649–657.

NEI, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA 70: 3321–3323.

NYBOM, H. AND B. A. SCHAAL. 1990. DNA "fingerprints" reveal genotypic distribution in natural populations of blackberries and raspberries (*Rubus*, Rosaceae). Amer. J. Bot. 77: 883–888.

OVERATH, R. D. AND M. A. ASMUSSEN. 1998. Genetic diversity at a single locus under viability selection and facultative apomixis: Equilibrium structure and deviations from Hardy-Weinberg frequencies. Genetics 148: 2029–2039.

PHIPPS, J. B., K. R. ROBERTSON, J. R. ROHRER, AND P. G. SMITH. 1991. Origins and evolution of subfam. Maloideae (Rosaceae). Syst. Bot. 16: 303–332.

PIELOU, E. C. 1969. An Introduction to Mathematical Ecology. Wiley-Interscience, New York.

PROCTOR, M. C. F., M. E. PROCTOR, AND A. C. GROENHOF. 1989. Evidence from peroxidase polymorphism on the taxonomy and reproduction of some *Sorbus* populations in south-west England. New Phytol. 112: 569–575.

RICHARDS, A. J. 1986. Plant Breeding Systems. George Allen & Urwin, London.

ROBINSON, W. A. 1982. Experimental taxonomy in the genus *Amelanchier*. II: Do taxa in the genus *Amelanchier* form an agamic complex? Rhodora 84: 85–100.

——— AND C. R. PARTANEN. 1980. Experimental taxonomy in the genus

Amelanchier. I: A new look at the chromosome numbers of the *Amelanchier* species growing in the northeastern United States. Rhodora 82: 483–493.

ROHLF, F. J. 1988. Numerical Taxonomy and Multivariate Analysis System. Exeter Publishing, Setauket, NY.

SHERMAN-BROYLES, S. L., J. P. GIBSON, J. L. HAMRICK, M. A. BUCHER, AND M. J. GIBSON. 1992. Comparisons of allozyme diversity among rare and widespread *Rhus* species. Syst. Bot. 17: 551–559.

SLATKIN, M. 1985. Rare alleles as indicators of gene flow. Evolution 39: 53–65.

SOLTIS, D. E., C. H. HAUFLER, D. C. DARROW, AND G. J. GASTONY. 1983. Starch gel electrophoresis of ferns: A compilation of grinding buffers, gel and electrode buffers, and staining schedules. Amer. Fern. J. 73: 9–27.

WEBER, J. E. AND C. S. CAMPBELL. 1989. Breeding system of a hybrid between a sexual and an apomictic species of *Amelanchier*, shadbush (Rosaceae, Maloideae). Amer. J. Bot. 76: 341–347.

WIEGAND, K. M. 1912. The genus *Amelanchier* in eastern North America. Rhodora 14: 117–161.

WRIGHT, S. 1951. The genetic structure of populations. Ann. Eugen. 15: 323–354.